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This is to certify the attached substitute specification contains no new matter.

Respectfully submitted,

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Device and Method for Carrying out Immunological Marking Techniques for Thin-Sectioned Tissue

The present invention concerns a device for treating thin-sectioned tissue on a support plate with at least one treatment liquid.

Moreover, the present invention concerns a method for treating thin-sectioned tissue on a support plate with at least one treatment liquid.

To be able to study the structure of biological specimens such as tissues or cells with an electron microscope, ultra-thin sections only a few nm in size are prepared and placed on object support nets made of metal, preferably of nickel (in the following referred to as small metal nets). For electron-microscopic studies, the sections are rendered contrasty or individual constituents of the specimen are marked by use of special or cytochemical methods. These cytochemical methods are often based on the principle of ligand pair formation, the first ligand possibly being present in the biological specimen and the second ligand, upon coming in contact with this specimen, binding as binding partner to the first ligand. Examples of biologically based ligand pairs are antigen/antibody binding pairs, enzyme/substrate binding pairs, lectin/sugar, hormone/receptor systems, DNA/DNA pairs and DNA/RNA pairs.

The prior art discloses numerous methods involving the antigen/antibody binding pair. These methods are collectively referred to as immunohistochemistry and immunocytochemistry (in the following referred to as marking techniques). For example, US Patent 5,143,714 discloses a method whereby an antigen is adsorbed from a liquid specimen into a pelletizable gel substance. The gel pellet is surrounded by a diffusion barrier, integrated as a block into a punched-out gel matrix and subsequently, as a tissues specimen, subjected to immunological marking techniques. DE 38 78 167 T2 describes the use of colloidal gold particles for the marking of ligands by the immunogold staining technique. A greatly improved method permiting qualitative and quantitative evaluation of an antigen in a specimen is disclosed in US Patent 5,079,172 as a sandwich assay whereby the antigen-binding first antibody is marked with a gold-labeled second antibody

that binds to the first antibody. By the electron-microscopic evaluation method, the antigen in the specimen can be determined qualitatively and quantitatively from the amount of gold particles.

Many procedures of immunohistochemistry and immunocytochemistry for immunological marking of thin-sectioned tissues have in common the feature that in most cases they consist of 10 to 20 individual processing steps. In a large number of cases, the processing steps consist of washing the specimen to be studied with buffering or marking solutions.

Such washing is currently carried out manually by an expensive procedure whereby individual drops of the aqueous buffer solution or marking solution are placed onto a hydrophobic substrate (for example Parafilm®, Parlodion®, Colloidion or Formfan®). The small metal nets with the thin-sectioned tissue are placed individually onto the drops to enable the tissue to react with the treatment liquid. Because of the light weight of the small metal net and the surface tension of the liquid drop, the small metal net floats on the surface of the drop. After a certain contact time for this step (often 5-10 min), the small metal net is moved on to the next drop by use of tweezers. This is continued all the way to the last position of the standard procedure tying down an operator for up to several hours per immunological marking reaction.

It can readily be seen that this manual process requires the continuous attention of the working personnel and that the labor costs are high because of the large amount of time involved. The number of specimens that can be worked on at the same time is very limited, and errors by the working personnel during the exact pipetting and positioning of liquid drops of very small volume cannot be excluded. The manual method cannot exclude mixing up the specimens after the long treatment period during the immunological marking, but this could be pre-vented by use of a specimen carrier with identification in the form of a chip or bar code, as described in Utility Model DE 299 06 382 U1.

Furthermore, the evaporation of the liquid drops during prolonged standard procedures presents a major problem.

Although Utility Model DE 298 17 912 U1 discloses a device for washing preparations to be subjected to microscopy on carriers following immunochemical treatment, this device is a washing box in which a major quantity of washing solution flows over the preparation and the carrier at a certain flow rate. Such a device is not suited for immunological marking techniques, because the antibody-containing marking solutions used are very expensive and, hence, are used in only very small volumes. To date, no device or method is known that allows an automated execution of immunological marking techniques for thin-sectioned tissues.

The object of the invention is therefore to provide a suitable device for the treatment of thin-sectioned tissues that makes it possible to process several thin-sectioned tissues effectively, nearly automatically and in time-saving and reproducible manner.

This objective is reached by use of a device comprising the features of claim 1.

Another object of the invention is a method for treating thin-sectioned tissues for the purpose of carrying out immunological marking techniques and that makes it possible to process several thin-sectioned tissues effectively, nearly automatically and in time-saving and reproducible manner.

According to the invention, this objective is reached by use of a method comprising the features of claim 22.

The device according to the invention consists essentially of two parts, a support plate on which small metal nets are placed and held with a magnet, and an object support provided with recesses (known as wells) that can be filled with at least one liquid (treatment liquids for immunological marking or washing solutions, both known from the prior art). The positions of the recesses correspond in their arrangement to the positions of oppositely located small metal nets on the

support plate. In one arrangement, both parts of the device can be introduced into a conveying device with controllable raising and lowering function in a manner such that the support plate with the small metal nets comes in contact with the liquid drop in the recesses of the object support from above, like a die. This procedure permits automatization of immunological marking methods for thin-sectioned tissues intended for electron-microscopic studies. Several object supports can be automatically assigned to a treatment position, and a multiplicity of small metal nets can be disposed on a single support plate, so that the number of specimens that are to be worked on simultaneously is markedly increased. The device for treating thin-sectioned tissues contains at least one support plate so that the thin-sectioned tissues can be treated with at least one treatment liquid. At least one object support in a treatment position is disposed opposite the support plate. Several object supports can be brought automatically into this treatment position. The support plate defines an underside on which the marked positions are indicated and where small metal nets with thin-sectioned tissues are positioned. The support plate also defines an upper side containing several holes each hole containing a wedged-in magnet. The holes are disposed opposite the marked positions. The object support defines an upper side containing free recesses configured for receiving the treatment liquid.

In the following, the invention will be explained by reference to the examples that are schematically represented in the drawings, in which:

Fig. 1 is a view in perspective of a support plate carrying small metal nets;

Fig. 2 is a view in perspective of an object support with recesses that can be filled with at least one treatment liquid;

Fig. 3 is a detailed cross-sectional view of the support plate and object support;

Fig. 4 is a top view of one embodiment of the object support;

Fig. 5 is a view in perspective of the conveying device for placing the support plate onto the object support;

Fig. 6 is a schematic representation of the support plate which with the aid of the conveying device is in approximate contact with an object support;

Fig. 7 is a schematic representation of the chamber for protection against evaporation of the liquid drops on the object supports, and

Fig. 8 is a schematic representation of another embodiment of the chamber for protection against evaporation of the liquid drops on the object support.

Fig. 1 shows a support plate 1 of the invention that defines an upper side 1a and an underside 1b. On underside 1b, support plate 1 is provided with marked positions on which are located small metal nets 2 with the thin-sectioned tissues (not shown here). Preferably, the positions of the small metal nets 2 on the support plate 1 are raised. This prevents the formation of liquid bridges between the individual small metal nets 2 when said nets make contact with the liquid drops. On the upper side 1a of support plate 1 are located, opposite the positions of small metal nets 2, holes 3a (see Fig. 3) in which are lodged magnets 3, for example, permanent magnets. Said magnets keep small metal nets 2 in their place on the underside 1b of support plate 1 by magnetic force. The distance between small metal nets 2 and magnet 3 should be as small as possible (distance < 2 mm). Support plate 1 preferably consists of dimensionally stable non-magnetic material, preferably aluminum, brass or a fiber-reinforced plastic material, and underside 1b is advantageously provided with a hydrophobic coating (for example, a Teflon compressive coating]. To a person skilled in the art, it is self-evident that numerous embodiments are possible for the shape of support plate 1 and for the arrangement of the small metal nets 2 on support plate 1. In the embodiment represented here, three small metal nets 2 are located on a rectangular plate with dimensions of about 76 x 26 mm (3 x 1 inch; object support size), said small metal nets having a diameter of 3 mm. It is critical that the positions of small metal nets 2 on the underside 1b of support plate 1 be located opposite the holes for magnets 3 on upper side 1a of support plate 1 and preferably also opposite the positions of at least one liquid drop 6 on upper side 4a of an object support 4 (see Fig. 2).

In another embodiment, small metal nets 2 are held on the underside 1b of support plate 1 by electromagnets (not shown).

Fig. 2 shows a view in perspective of the object support 4 of the invention that defines an upper side 4a and an underside 4b. On upper side 4a, object support 4 is provided with several recesses 5 (known as wells) which in the embodiment shown are arranged in a line and each is filled with a liquid drop 6. Liquid drop 6 consists of a washing solution or a treatment solution as disclosed in the prior art. It is also possible for individual recesses 5 to contain different liquids (for example one line of recesses 5 filled with washing solution, the next line of recesses 5 filled with marking solution). Advantageously, object support 4 is transparent and is made of dimensionally stable material. Object support 4 is preferably made of glass or a plastic material, the upper side 4a of said support that carries recesses 5 and the recesses 5 themselves being covered with a hydrophobic coating (for example, with a Teflon compressive coating 5a). Recess 5 is identical with thickness D (see Fig. 3) of the Teflon coating 5a. Recess 5 preferably amounts to about 50 μm. Recesses 5 hold a liquid volume which for washing solution is about 50 μL and for antibodies and gold solutions about 5 μL. The Teflon compressive coating is the same for different drop sizes. Because of the hydrophobic boundary layer, the liquid drops 6 are more or less curved upward. To bring the small nets in contact with these drops, it is absolutely necessary for the operation that one know the drop size, because a difference in drop size will require a different position for the lowering movement of small metal nets 2.

Fig. 3 shows a partial cross-sectional view of the relative spatial relationship of object support 4 to support plate 1. The partial view of the cross-section is defined, for example, in Fig. 2 by broken line 32. In the embodiment shown here, object support 4 has applied to it a hydrophobic coating 5a having thickness D. In support plate 1, there are provided holes 3a for magnets 3. On the underside 1b of support plate 1 are present marked positions 2a each of which carries one small metal net 2. Small metal net 2 is held by magnets 3 assigned to marked positions 2a. The dash-and-dot line 29 in Fig. 3 shows clearly that support plate 1 and object support 4 are disposed in a manner such that one marked position 2a with a small metal net 2 is located opposite a recess 5,

Fig. 4 shows a top view of object support 4. This is a further embodiment of object support 4. For identification, object support 4 is advantageously provided with an identification as described, for example, in Utility Model DE 299 06 382 U1. Identification 4c is located on surface 4a of object support 4 and can be in the form of a bar code or a chip. To a person skilled in the art it will be obvious that numerous embodiments are possible for the size and shape of object support 4 as well as for the arrangement of recesses 5 on upper side 4a of object support 4. On a rectangular surface with dimensions of 76 mm x 26 mm, there can be disposed, for example, 10 x 3 recesses 5 with a size from 2 mm to 3 mm. It is critical that the positions of liquid drops 6 on upper side 4a of object support 4 be disposed opposite the positions of small metal nets 2 on the underside 1a of support plate 1 (see representation in Fig. 3).

Fig. 5 shows support plate 1 of the invention in a conveying device 20 with controllable raising and lowering function. Conveying device 20 is designed so that several object supports 4 can successively be brought in contact with support plate 1. Conveying device 20 is controlled so that the positions of recesses 5 of object support 4 containing liquid drops 6 are located exactly opposite the positions of small metal nets 2 on the underside 1b of support plate 1. To this end, by means of a motor 21 of conveying device 20, support plate 1 is centered from above, parallel and sideways, until the positions of small metal nets 2 and those of liquid drops 6 are located exactly opposite each other. Conveying device 20 then brings support plate 1 closer to object support 4. For this purpose, on conveying device 20 there is provided a track 22 that lowers support plate 1 linearly onto object support 4, said support plate being held by means of a holder 9 in a holding head 23 of conveying device 20. Object supports 4 are placed, for example, on a conveyor 24 that moves the object supports 4 in an appropriate manner toward conveying device 20. Conveying device 20 and conveyor 24 are controlled and guided by a computer unit 25 and an appropriate computer program.

Fig. 6 shows a schematic representation of the support plate 1 of the invention which by conveying device 20 (see Fig. 5) is brought into approximate contact with object support 4. On its underside 1b, support plate 1 is provided with

marked positions 2a at which are located in raised position small metal nets 2 with thin-sectioned tissues 2b. On upper side 1a of support plate 1 are located opposite the positions of small metal nets 2 holes 3a in which are lodged magnets 3 so as to keep small metal nets 2 in their place on underside 1b of support plate 1 by magnetic force.

At a certain small distance between underside 1b of support plate 1 and the upper side 4a of object support 4, liquid drops 6 wet the thin-sectioned tissues 2b on small metal nets 2. This distance depends on the volume of liquid drop 6 and varies from 0.5 mm to 4 mm. The transport of support plate 1 and object support 4 is accomplished by linear guidance provided by a stepping motor (not shown). The positions of object support 4 and support plate 1 are monitored by position sensors 7 and correspondingly controlled. The signals from the various position sensors 7 are used for control purposes. In the embodiment shown here, there are provided vertical, parallel guidance tracks 8 by means of which holding head 23 (see Fig. 5) together with support plate 1that is fixed in a holder 9 can be lowered onto object support 4. Holder 30 fixes support plate 1 and one object support 4 relative to each other in a treatment position 14 (see Fig. 5). In this case, small metal nets 2 carrying a thin-sectioned tissue 2b are in contact with liquid drops 6 on the upper side 4a of object support 4. After a preset incubation time, a change of support plate 1 to the next arrangement of liquid drops 6 on the same object support 4 or on the next object support 4 is brought about automatically. To this end, support plate 1 is automatically lifted, and object support 4 is transported one position further. Arrow A in Fig. 6 indicates the vertical transport direction of support plate 1, while arrow B shows the horizontal transport direction of object support 4.

The subsequent course of the transport of object support 4 is not described in detail, but it can occur in various ways. Those skilled in the art know, for example, the following arrangements: Object supports 4 are placed on a linear conveyor 24 moved by a motor 26. Alternatively, object supports 4 are placed on a circular conveyor 24 also moved by a motor.

To prevent a reduction in volume of liquid drops 6 by evaporation, object supports 4 on conveyor 24 are provided with a cover 10 so as to form a chamber 11. In this chamber 11, high air humidity is attained, for example, by inserting moist filter paper 13 on a holder 12, the evaporation of liquid drops 6 thus being minimized.

Fig. 7 shows a schematic representation of chamber 11 as evaporation protection for liquid drops 6 on object support 4. In the chamber, a stack of moist absorbent paper 13 is held on a holder 12. Before liquid drops 6 come in contact with small metal nets 2, cover 10 is automatically opened, at least partly.

Fig. 8 shows a schematic representation of another embodiment of chamber 11 as evaporation protection for liquid drops 6 on object support 4. In a diferent embodiment, chamber 4 is designed, for example, so that the moist filter paper 13 is located in chamber 10 below object support 4. In this embodiment, several chambers 10 are stacked one on top of another, the next chamber being placed on the chamber below it thus closing it. The moisture of filter paper 13 passes by object support 4 from below and laterally and then enters chamber 10. Object support, so to speak, thus rests on stilts 28 in a pool. The top-most chamber 10 is finally closed off with a cover 27. Object supports 4 are transported to treatment position 14 in an appropriate manner. In the treatment position, support plate 1 is brought in contact with the object support.

The device of the invention is so configured that it carries out automatically the following steps of an already existing standard procedure for immunological marking of thin-sectioned tissues: saturating non-specific bonds, antibody incubation and diverse washing steps with different washing solutions.

In another embodiment, in the treatment position there is provided a heat source, for example in the form of a small electric resistance device, or a cooling unit, for the purpose of adjusting the optimum reaction temperature of object support 4 and liquid drops 6. Said heat source is configured so that an electric contact of the object carrier triggers the heating or cooling device of said object support. The heat source or cooling unit is controlled by means of temperature sensors disposed on object support 4 or in the vicinity of the liquid drop and is regulated

by the attached computer unit 25 and an appropriate computer program. The heating or cooling of object supports 4 can also occur by contact of object supports 4 with a temperature-regulated metallic plate 31.

A further object of the invention is a method whereby, by use of the described device, several small metal nets 2 with thin-sectioned tissues 2b resting thereon are treated on a support plate 1, particularly to carry out immunological marking and/or washing steps reproducibly, at the same time, effectively and in time-saving manner. The automated procedure reduces the human source of error.

The method optimizes the treatment, particularly the carrying out of immunological marking and/or washing steps for thin-sectioned tissues for electron-microscopic specimens. The individual procedures concerning the kind of liquid and incubation time can be found in the prior art.

Instead of transporting small metal nets 2 with tweezers from one liquid drop 6 to the next one, thin-sectioned tissues 2b are positioned on small metal nets 2 and placed on support plate 1 of the invention which is provided with marked and raised positions 2a and to the underside 1b of which has advantageously been applied a hydrophobic coating. Magnets 3 located on the upper side 1a of support plate 1 hold the small metal nets 2 in their place by magnetic force (see Fig. 6).

Support plate 1 is held by a conveying device 20 with controllable raising and lowering function (see Fig. 5) and lowered

With a microliter pipette, liquid drops 6 of an exact volume are placed into recesses 5 of object support 4 of the invention. In the treatment position 14, the positions of the liquid drops 6 on object support 4 are located exactly opposite the positions of small metal nets 2 on support plate 1 (see Fig. 6).

Object support 4 is placed on conveyor 24 of conveying device 20. The adjustment of the object support 4 and of liquid drops 6 to a certain temperature

is done by means of computer unit 25 through a heat source or cooling unit (not shown) which are triggered by an electric contact of the object support.

Conveying device 20 and conveyor 24 cooperate with one another in that support plate 1 with small metal nets 2 is centered parallel to and laterally relative to object support 4. The positions of small metal nets 2 and of liquid drops 6 are located exactly opposite each other. Cover 10 which protects liquid drops 6 from evaporation and attendant volume reduction in a chamber 11 with moist absorbent paper 13 is automatically at least partly opened. At a certain small distance between support plate 1 and object support 4, liquid drops 6 wet the small metal nets 2. This position is now held constant for the particular programmed incubation time. The distance depends on the drop volume and varies from 0.5 mm to 4 mm. The motor driven transport mechanisms of conveyor 24 and conveying device 20 are controlled by computer unit 25, a computer program and position sensors 7.

At the end of the selected incubation time, support plate1 automatically moves over to the next liquid drop 6. Support plate 1 is automatically lifted by use of conveying device 20 and sensor 7, and object support 4 is automatically transported further by one position, or an object support 4 with liquid drops 6 is automatically introduced into a carousel, a cartridge or a stack from a linear guide with several object supports.

By this method, the following steps of a standard procedure for immunological marking of thin-sectioned tissues from the prior art with several small metal nets 2 and thin-sectioned tissues 2b resting thereon are carried out automatically and reproducibly: saturation of non-specific bonds, antibody incubation and diverse washing steps with different washing solutions.

The invention was described by reference to a particular embodiment. It will be obvious to a person skilled in the art, however, that derivations and modifications thereof can be performed without exceeding the protective scope of the claims.

List of Reference Numerals

1	Support plate
1a	Upper side of support plate
1b	Underside of support plate
2	Small metal nets
2a	Marked positions on support plate
2b	Thin-sectioned tissue
3	Magnets
3a	Holes for magnets
4	Object support
4a	Upper side of object support
4b	Underside of object support
4c	Identification on object support
5	Recesses (wells)
5a	Teflon coating
6	Liquid drops
7	Position sensors
8	Vertical guiding tracks of conveying device
9	Holder for support plate
10	Cover of object support
11	Chamber
12	Holder for absorbent paper
13	Stack of moist absorbent paper
14	Treatment position
20	Conveying device
21	Motor of conveying device
22	Guiding track of conveying device
23	Holding head
24	Conveyor
25	Computer unit
26	Motor of conveyor
27	Cover

28	Stilts
29	Dash-dot line
30	Holder for conveying device
31	Temperature-regulated plate
32	Broken line
A	Arrow indicating vertical transport direction
B	Arrow indicating horizontal transport direction
D	Thickness